

Adnp Cas9-CKO Strategy

Designer:Fengjuan Wang

Reviewer:Shilei Zhu

Design Date:2020-2-27

Project Overview

Project Name

Adnp

Project type

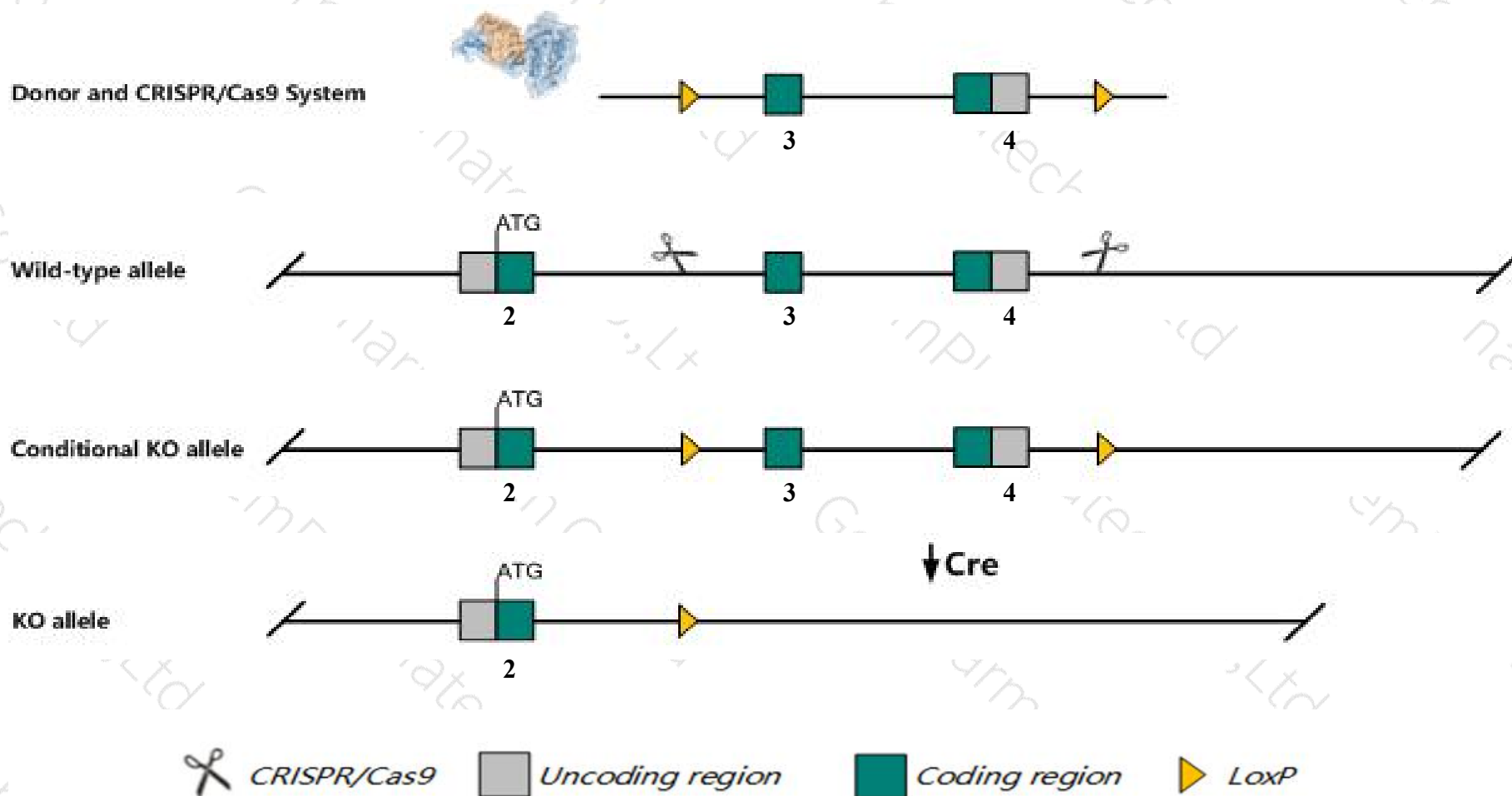
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Adnp* gene. The schematic diagram is as follows:



- The *Adnp* gene has 4 transcripts. According to the structure of *Adnp* gene, exon3-exon4 of *Adnp*-202 (ENSMUST00000088001.5) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Adnp* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- According to the existing MGI data, Developmental defects including the failure of the cranial neural tube to close lead to embryonic death between E8.5 and E9.
- The *Adnp* gene is located on the Chr2. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Adnp activity-dependent neuroprotective protein [Mus musculus (house mouse)]

Gene ID: 11538, updated on 17-Feb-2019

Summary



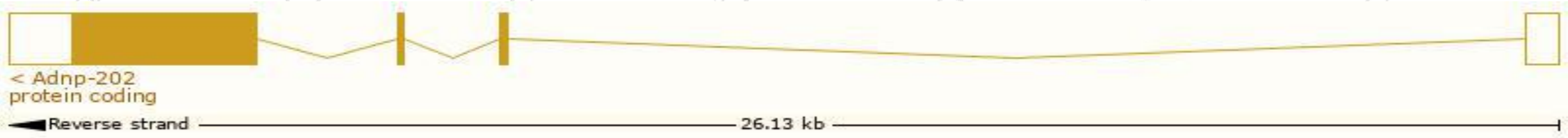
| | |
|---------------------------|---|
| Official Symbol | Adnp provided by MGI |
| Official Full Name | activity-dependent neuroprotective protein provided by MGI |
| Primary source | MGI:MGI:1338758 |
| See related | Ensembl:ENSMUSG00000051149 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | AA589558, mKIAA0784 |
| Summary | This gene encodes a member of a protein family characterized by nine zinc finger motifs followed by a homeobox domain. In vitro studies demonstrate that the encoded protein interacts with the brahma-related gene1-associated or hBRM factors (BAF) gene expression regulating complex, components of the protein translation machinery, and microtubule-associated proteins. This gene has been implicated in neuroprotection through various processes that include chromatin remodeling, splicing, cytoskeletal reorganization, and autophagy. Homozygous mutant knockout mice display embryonic lethality with defects in neural tube closure. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Nov 2016] |
| Expression | Ubiquitous expression in CNS E11.5 (RPKM 36.2), CNS E14 (RPKM 35.6) and 28 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

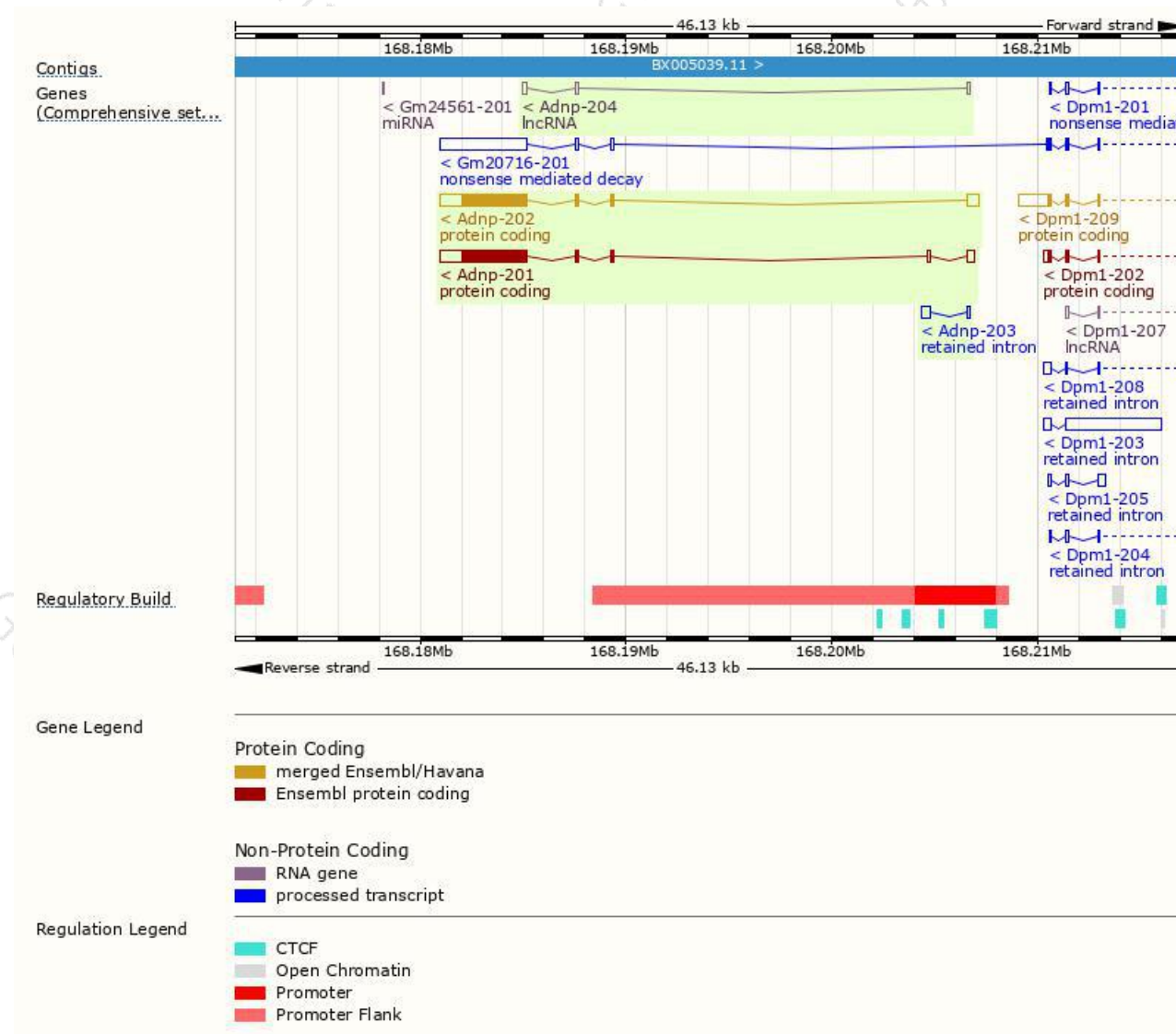
The gene has 4 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|---------------------------------------|------|------------------------|-----------------|---------------------------|------------------------|-------------------------------|
| Adnp-202 | ENSMUST00000088001.5 | 4932 | 1108aa | Protein coding | CCDS38342 | Q9Z103 | TSL:1 GENCODE basic APPRIS P1 |
| Adnp-201 | ENSMUST00000057793.10 | 4917 | 1108aa | Protein coding | CCDS38342 | Q9Z103 | TSL:5 GENCODE basic APPRIS P1 |
| Adnp-203 | ENSMUST00000131200.1 | 554 | No protein | Retained intron | - | - | TSL:2 |
| Adnp-204 | ENSMUST00000139984.7 | 434 | No protein | lncRNA | - | - | TSL:2 |

The strategy is based on the design of *Adnp-202* transcript,The transcription is shown below



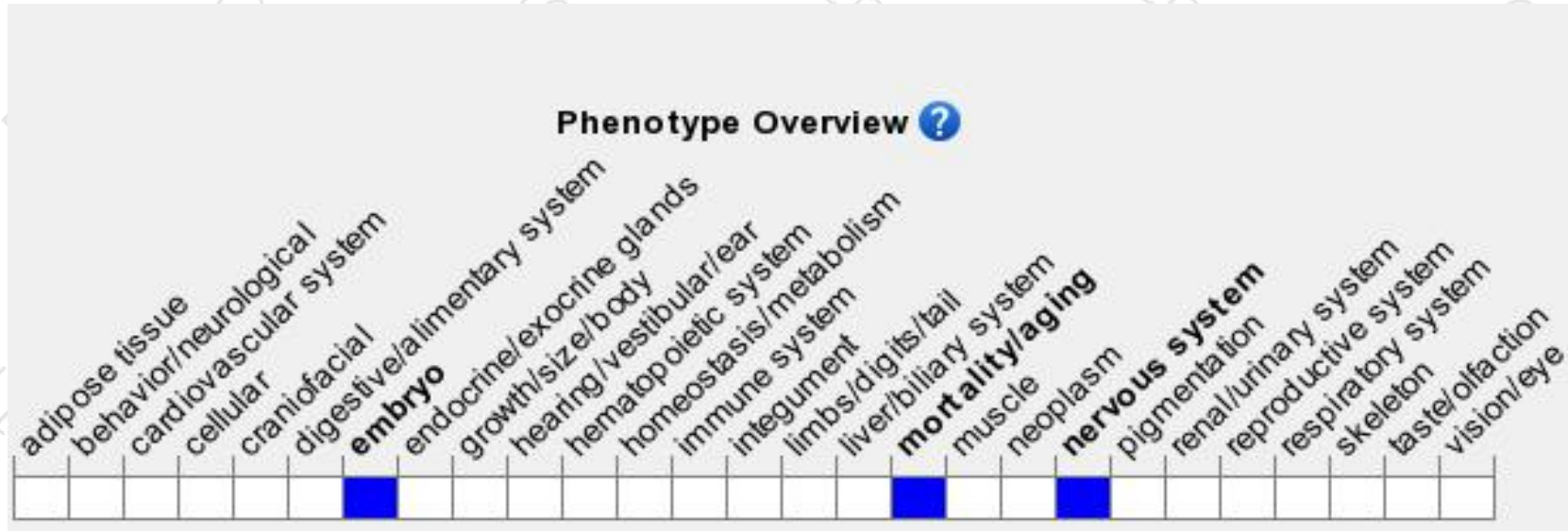
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, Developmental defects including the failure of the cranial neural tube to close lead to embryonic death between E8.5 and E9.

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

